CHAPTER 2
BACKGROUNDS AND LITERATURE REVIEWS

2.1 Background

2.1.1 History of Hexachlorobenzene

Hexachlorobenzene (C_6Cl_6; HCB) does not happen naturally in the environment. Its main source is the manufacture of pesticides and it is an unwanted by-product in the manufacture of solvents and chlorinated compounds. HCB is also used as a chemical intermediate in dye manufacture and synthesis of other organic chemicals, in the production of pyrotechnic compositions for the military, as a raw material for synthetic rubber, a plasticizer for polyvinyl chloride, and as a wood preservative (ATSDR, 2000; HSDB, 2001).

HCB was listed as one of the twelve persistent organic pollutants (POPs) in the Stockholm Convention for its tendency to accumulate along the food chain and its recalcitrance to degradation, together with its harmful effects on human beings and the environment. Microbial degradation is a promising effective way to bioremediation environmental pollutants, including persistent organic pollutants. HCB is very toxic to aquatic organisms. It may cause long term adverse effects in the aquatic environment. Therefore, release into waterways should be avoided. It is persistent in the environment. Ecological investigations have found that biomagnifications up the food chain does occur. HCB has a half-life in the soil of between 3 and 6 years (ATSDR, 1994). HCB may cause long term adverse effects in the aquatic environment. Risk of bioaccumulation in an aquatic species is high. Primarily an antifungal pesticide, HCB has been out of commercial use in the United States since 1965 and is banned in many countries. In Thailand, HCB is a one of 84 pesticides that have been banned since October, 2001. Yet new HCB still enters the environment as a by-product of several activities, including the manufacture of other chemicals, wood preservation and the burning of municipal garbage. It can build up in wheat, grasses, and some vegetables. HCB is also found concentrated in fish, marine mammals, birds, and lichens, as well as in grazing animals that eat lichens or grass, and in animals that eat fish. It turns up in the human food supply in contaminated dairy products, meat, and fish (Jensen and Slorach, 1991). Low levels of HCB have been identified in the fatty tissue of almost all people

tested, according to the Agency for Toxic Substances and Disease Registry (ASTDR, 1997).

2.1.2 Chemical and Physical Properties

HCB, with the properties as shown in Table 2.1 and its molecular structure as shown in Figure 2.1, is a white crystalline solid, hydrophobic, strongly absorb to soil/sediment organic matter and tend to persist in soils. It is insoluble in water, but is soluble in benzene, carbon disulfide, chloroform, and ether. Under most environmental conditions, it has a very low degradation rate. It is combustible, but it does not ignite readily. When heated to decomposition, HCB emits highly toxic fumes of hydrochloric acid, other chlorinated compounds, carbon monoxide, and carbon dioxide. HCB is a stable and nonreactive compound, but it can react violently with dimethylformamide at temperatures higher than 65°C. The technical grade product contains 98% HCB, 1.8% pentachlorobenzene (QCB), and 0.2% tetrachlorobenzene (TeCB) (HSDB, 2001). It has low water solubility (in water at 25°C: 0.005 mg/L), moderate volatility (vapor pressure at 20°C: 1.09×10^{-5}, Henry's law constant at 25°C: 5.8×10^{-4} atm-m^3/mol) and thus is likely to show low mobility in the soil environment (ATSDR, 2002). Principle chemical and physical properties of HCB are shown in Table 2.1.

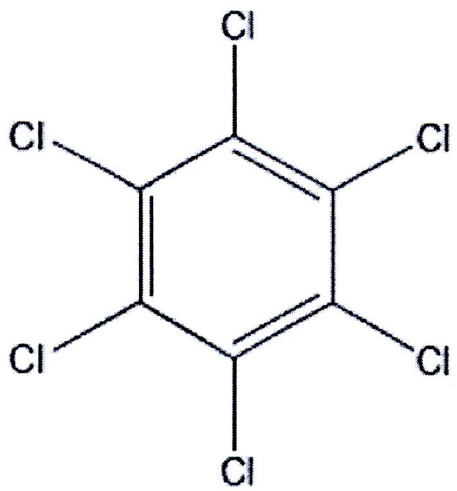

Figure 2.1 The structure of hexachlorobenzene

Table 2.1 Properties of hexachlorobenzene. (ATSDR, www.atsdr.cdc.gov, 2002)

Common name	hexachlorobenzene
CASRN (Chemical Abstract Service Registry Number)	118-74-1
Chemical name	hexachlorobenzene
Other name[a]	amatin anticarie benzene hexachloride bunt-cure bunt-no-more ceku C.B. co-op hexa esclorobenzene granox nm HCB hexa c.b. hexachlorbenzol 1,2,3,4,5,6- hexachlorobenzene julen's carbon chloride julin's carbon chloride no bunt no bunt 40 no bunt 80 no bunt liquid pentachlorophenyl chloride perchlorobenzene phenyl perchloryl Saatbeizfungizid sanocid Sanocide smut-go Snieciotox
Molecular formula	C_6Cl_6
Relative molecular mass	284.79
Melting Point (°C)	231
Boiling Point (°C)	323-326 (sublimates)
Flash Point (°C)	242
Density (g/cm^3 at 20°C)	1.5691
Vapor Pressure (mm Hg @ 20°C)	1.09×10^{-5}
Water Solubility (mg/L)	0.0058 (at 20 °C) 0.0062 (at 25 °C)
n-octanol-water partition coefficient, K_{ow} (log K_{ow})	5.5
Henry's Law Constant (calculated)[b] ($Pa/mol/m^3$)	131
Conversion factors	1 ppm = 11.84 mg/m^3 1 mg/m^3 = 0.08 ppm

Note: [a]: from Chemfinder, 1999; IPCS, 1997; NIST, 1999]

[b]: Henry's Law Constant has calculated using the tabled values for aqueous solubility and vapor pressure.

2.1.3 Uses of HCB

Historically, HCB was widely used as a seed dressing for prevention of fungal growth on crops such as wheat, barley, oats and rye. Concern for adverse affects to the environment and human health resulted in the discontinued use of HCB as a pesticide in many countries during the 1970s. However, additional uses for HCB continued beyond this period including its application in fireworks, ammunition, and synthetic rubbers. Recent data regarding production levels of HCB are limited. Worldwide production of pure HCB was estimated to be 10,000 tons/year from 1978 to 1981. An estimated 300 tons were produced by three manufacturers in the United States in 1973 (IARC, 1979), while approximately 1500 tons of HCB were manufactured annually in Germany for production of the rubber auxiliary PCTP. This latter use was discontinued in 1993. While restrictions initiated in the 1970s resulted in the decline of HCB manufacturing, it continues to be produced as an unintentional by-product in the manufacturing of chlorinated solvents, aromatics and pesticides. In the 1980s, an estimated 4,130 tons of HCB were generated annually as a by-product in the production of chlorinated organic and pesticides. In the United States, the majority of this is due to the manufacture of chlorinated solvents such as carbon tetrachloride, trichloroethylene and perchloroethylene. HCB is also inadvertently produced through incineration of HCB-containing materials, which constitutes a further source of entry into the environment. While emission levels from incinerators are considered site-specific, crude estimates of total HCB release from municipal incinerators in the United States have been estimated to be between 310 and 977 kg/year.

2.1.4 Environmental Fate

HCB may enter the environment through air emissions and in wastewater from facilities involved in the production of several chlorinated solvents or use of pyrotechnic mixtures. Once in the environment, HCB will primarily exist in the vapor phase and degradation is extremely slow. With its resistance to environmental degradation and mobility, HCB is widely distributed throughout the environment. HCB undergoes limited atmospheric photolytic degradation with a half-life of around 80 days. As a result, long-distance atmospheric transport is possible and is considered to play a significant role in its wide environmental distribution. Wet deposition via rain and snowfall are believed to be the primary means of HCB transfer from the atmosphere to

aquatic and terrestrial systems in Canada. In water, HCB will evaporate rapidly (half-life of 8 hours) due to the moderate Henry's Law constant, adsorb to sediments, or bioconcentrate in fish and other aquatic organisms. Once in the sediment, HCB tends to become trapped by overlying sediment. Desorption from soil and sediment although limited, does occur and is a potential continual source of HCB re-entry into the environment, even after other inputs have been discontinued. In soil, volatilization is the primary means of removal at the surface interface. Aerobic ($t_{1/2}$ of 2.7-5.7 years) and anaerobic ($t_{1/2}$ of 10.6-22.9 years) biodegradation are the major means of HCB removal at lower soil depths.

2.1.4.1 Air

While HCB is widely dispersed in air, concentrations are generally low. Reported HCB air levels are generally similar at urban, rural and remote sampling sites. Typical mean concentrations of HCB in air collected from point sources in Canada, Norway, Sweden, Germany, the United States, the Arctic, and the Antarctic range from 0.04 to 0.6 ng/m^3 Similar low levels were detected in the non-occupational pesticide exposure study conducted by the U.S. EPA in the mid to late 1980s. HCB was detectable in 6 to 45 percent of the samples collected in Jacksonville Florida at concentrations ranging from below detection to 21 ng/m^3. The mean concentration for the sampled homes was 0.4-0.9 ng/m^3. In contrast, HCB was rarely detected in Springfield/Chicopee Massachusetts, the other area of study, and the levels were very low (<0.1 ng/m^3). Interestingly, the levels detected in indoor air were consistently higher than those measured in outdoor air. Occasionally, other studies have reported higher values for HCB in the air. For example, Grimalt et al. (1994) reported an average airborne HCB concentration of 35 ng/m^3 in the vicinity of an organochlorine factory in Catalonia, Spain, compared with an average of 0.3 ng/m^3 for Barcelona, a nearby reference city. Spigarelli et al. (1986) detected HCB in the air at concentrations as high as 24 µg/m^3 at an on-site landfill of a manufacturer of perchloroethylene, carbon tetrachloride and chlorine.

2.1.4.2 Water

In surface waters, HCB strongly adsorbs to sediment and suspended matters, resulting in a wide range of concentrations in water and sediment due to differences in water levels, sediment composition and suspended matter. From 1987 to 1993, HCB release to

surface water was estimated at 1,286 pounds according to the U.S. EPA's Toxic Release Inventory. This release was primarily from alkali, chlorine and agricultural chemical industries. The largest releasing was reported from Louisiana and Texas. However, HCB levels in fresh water in Europe and North America were quite low, typically below 1 ng/L values have been reported for aquatic systems that receive direct industrial discharge and surface run-off. For example, the channels connecting the Great Lakes in Canada are often found to exceed HCB levels of 1.0 ng/L. Levels in the St. Clair River near the Dow Chemical outfall were as high as 87 ng/L in 1985 and 75 ng/L in 1986. HCB is infrequently detected in drinking water, and when measurable, the levels are typically at very low concentrations. Drinking water samples collected in 1980 from Canadian cities located near Lake Ontario ranged between 0.06 and 0.20 ng/L. In most other Canadian and United States surveys, HCB has not been detected. For California, this is also true, i.e., HCB was not detected in 6,095 wells from 1984 to 2001. However, of the 17 public water supplies monitored and reported recently in National Drinking Water Contaminant Occurrence Database (NCOD), HCB was detected in one large system in Massachusetts at a reported concentration of 1.2 ng/L. A monitoring of higher concentrations of HCB (median of 1-2 ng/L) was reported in Croatian drinking water obtained from a polluted river.

2.1.4.3 Soil/Sediment

Limited data regarding HCB levels in soil have been reported. One of the most thorough data sets for HCB soil levels in the United States was developed from the 1972 US National Soils Monitoring Program in which 1,486 soil samples from 37 states were analyzed for organochlorines, organophosphates, PCBs, and elemental arsenic. HCB was detected in samples from 11 sites at concentrations ranging from 10 to 440 μg/kg dry weight. Sampling sites located near industrial sources typically contain the highest levels of HCB. Measurements as high as 12.6 μg/g were reported at a Canadian landfill site, and 5700 μg/g in the loading and transfer area of a plant manufacturing chlorinated solvents that used offsite disposal methods. Mean concentrations of HCB reported for uncontaminated soil in Europe ranged from 0.3 ng/g in Switzerland to 5.1 ng/g in a Swedish rural heartland soil.

2.1.5 Exposure

Current potential for exposure to HCB for the general population is limited because commercial production of HCB has ceased in many countries including Thailand. Nevertheless, it continues to be produced as a by-product from the manufacture of other chlorinated chemicals and persists in the environment from past releases. The production and use of HCB as a fungicide prior to 1984, and it occurrence as a by-product in the manufacture of other chemicals indicate that some human exposure may occur in both occupational and no occupational settings. Human exposure may occur through ingestion, inhalation and skin contact. Populations with potentially high exposure include chemical workers, individuals living near a waste site or industrial facility that may release HCB to the air or drinking water supplies, and individuals who ingest contaminated fish and wildlife (ATSDR, 2000; HSDB, 2001). The National Occupational Hazard Survey conducted by NIOSH (1976) from 1973 to 1974 estimated that 4,400 workers were possibly exposed to HCB in the workplace. The National Occupational Hazard Survey conducted from 1981 to 1983 indicated that 1,038 workers employed at 10 facilities were potentially exposed to HCB (ATSDR, 2000). Occupations with the highest potential for human exposure included fungicide application, organic chemical synthesis, synthetic rubber production, seed disinfection, pesticide, and wood preservation. HCB is among the most persistent environmental pollutants because of its relative stability and resistances to degradation. HCB released to the environment it taken up by plants and animals can build up through the food chain (ATSDR, 2000).

2.1.6 Impact of HCB

2.1.6.1 Health Impact

HCB can enter the body when consuming HCB-contaminated food, breathing HCB particles in the air, and/or when the skin comes in contact with it. Following intake, HCB rapidly spreads to many tissues in the body, especially to fat, probably within a few hours. Most of the HCB leaves the body in the feces, and smaller amounts are found in the urine. HCB will remain in the body, especially in fat, for years. A large portion of HCB in fat can be transferred in human milk. For short-term impact, EPA has found HCB to potentially cause skin lesions, nerve and liver damage to whom exposed at levels above the maximum concentration level (MCL) for relatively short periods of time. For long-term impact, HCB has the potential to cause damage to liver and

kidneys, reproductive organs, benign tumors of endocrine glands, and cancer to people who has a lifetime exposure at levels above the MCL.

2.1.6.2 Environmental Impact

HCB has been detected in environmental samples from around the world, and is recognized as a global pollutant. HCB is a highly persistent compound, with reported field half-lives in the soil environment up to 7.5 years. Evaporation is rapid while it is on soil surfaces, but considerably less so when it is mixed into the soil. HCB is moderately to strongly binded by most soils. Data from testing on hydro-soils indicate that it may be degraded both aerobically and anaerobically. It has low water solubility, and thus is likely to show low mobility in the soil environment. Due to its lengthy persistence, however, even low mobility may result in appreciable travel; therefore, HCB may pose some risk of groundwater contamination. HCB has been found in well water at low concentrations, up to 5.6 ppb and only in a very small percentage of all of the wells tested in the US. HCB is of low water solubility, so it would most likely reach surface waters via surface run-off by attachment to soil particles. Once in the aquatic environment, it is likely to be short-lived; HCB underwent very rapid, almost complete (that is, less than 5 days) degradation to pentachlorophenol and related compounds in inoculated hydro-soil samples under both aerobic and anaerobic conditions. Breakdown in vegetation appears rapid, with residue levels in grass at approximately 1% of the initial amount after 15 days, and at approximately 0.01% after 19 months. The dominant chemical loss process for gas phase HCB in the troposphere is by reaction with the hydroxyl radical (OH•). Based on this reaction, the atmospheric half-life is calculated to be about 1.4 years.

2.1.7 Toxicity

HCB is slightly toxic to practically nontoxic via the oral route of exposure. The reported acute oral LD_{50} values are 3500 mg/kg in the rat, 4000 mg/kg in the mouse, 2600 g/kg in the rabbit, and 1700 mg/kg in the cat (Edwards et al., 1991). Its toxicity via the dermal route has not been determined (Edwards et al., 1991; ATSDR, 1994). It is reported to be a possible skin irritant (Edwards et al., 1991). HCB is slightly to moderately toxic via inhalation, with reported inhalation LC_{50} values of 1.6 mg/L for the cat, 3.6 mg/L for the rat and 4 mg/L for the mouse. A syndrome called "porphyria"

is associated with HCB exposure as well. HCB is one of the most effective compounds at inducing porphyria in humans and in animals. Six of HCB-induced porphyria symptoms consist of blistering/scarring of the skin, light sensitivity, susceptibility to skin infection, and possibly osteoporosis (decreased bone calcium content). Pophyria is a general disruption in the normal metabolism of porphyrin compounds (often an over production), of which hemoglobin, and its chemical building blocks, are members. It is recognizable by the measurable increases in porphyrin compounds in the body and bodily fluids (e.g., blood, urine, feces, etc.) and increased activity of liver enzymes determined (Edwards et al., 1991; ATSDR, 1994).

2.1.7.1 Acute Toxicity

A search of the available literature failed to identify reports of acute toxic effects. However, several reviews have been published of an accidental poisoning incident in Turkey that occurred in 1955-1959 as a result of HCB-treated wheat grain being ground into flour and made into bread. The affected individuals consumed HCB-treated wheat seed originally intended for agricultural usage that was improperly diverted for food. During the incident, an estimated 3,000 to 5,000 people acquired a mixed porphyria resembling porphyria cutanea-tarda (PCT) (sometimes referred to as porphyria turcica) following consumption of an estimated 0.05 to 0.2 g of HCB per day per person. The period between the initial ingestion of the treated wheat and the onset of disease was estimated to have been 6 months.

2.1.7.2 Developmental and Reproductive Toxicity

A follow-up study of the Turkish women who were exposed to HCB in the 1950s was conducted approximately 40 years later to determine adverse reproduction outcome. Three groups were compared in this retrospective cohort study; one group of women previously exhibiting PCT, a control group from the region and a control from the Turkish capital of Ankara. The group of women previously exhibiting PCT reported a small decrease in the percentage of live births as compared to the other comparison groups. The other reproductive outcomes measured did not differ consistently between the three groups. The authors concluded that the induction of porphyria was not associated with adverse reproductive outcomes when measured 40 years after exposure. As part of the study, serum levels of HCB were measured in all three groups. The previously exposed women in this study did not report evidence of premature ovarian

failure nor were associations seen between serum FSH, estradiol or inhibin levels and serum HCB. Interestingly, a significant association between the concentration of HCB in the serum and the risk for spontaneous abortion was seen when the analysis was conducted using the combined values from all three groups. In a recent study in Germany, HCB concentration in neonatal serum correlated with maternal age ($r = 0.249$; $p < 0.01$), with 2.7-fold higher serum levels in offspring of 40 year-old as compared with 20-year-old women. The authors concluded that the neonatal burden depends on maternal age and duration of pregnancy. This reflected the increase in body accumulation with these substances during human life as well as continuous transplacental transfer from mother to fetus during pregnancy in a follow up, Lackmann, (2002) reported that neonatal blood levels of HCB have greatly decreased over the last 15 years. This is presumably due to decreased use of HCB and decreasing maternal exposures.

2.1.7.3 Chronic Toxicity

A variety of non-neoplastic effects have been observed in experimental animals resulting from chronic exposure to HCB. The effects primarily observed at the lowest dose levels are hepatic in nature, although effects to the kidney, spleen, and thyroid have been observed. Arnold et al. (1985) conducted a two generation study in which Sprague-Dawley rats were dosed by incorporating HCB into the feed at 0.32, 1.6, 8 and 40 ppm. Using the body weight and food consumption data supplied by the authors, U.S. EPA estimated the time weighted average dose levels received by male rats as 0.01, 0.05, 0.27 and 1.39 mg/kg-day, and by female rats as 0.01, 0.07, 0.35 and 1.72 mg/kg-day (U.S. EPA, 1985). The study design included in utero and lactational exposure prior to weaning by dosing both parents of the test animals. The test animals (50 per group) were subsequently dosed and observed for their whole lives. A control group received feed without HCB. There were no treatment-related effects on growth, feed consumption, hematological parameters or survival in either generation. Increased heart and liver weights were observed in the 8 and 40 ppm male treated with HCB for 3 months. HCB had no effect on fertility but pup viability was significantly reduced in the 40 ppm group.

2.1.7.4 Carcinogenicity

HCB has not been shown to be carcinogenic in humans. However, the epidemiological studies to date have not been designed to measure increases in cancer incidence and are therefore inadequate for risk assessment. HCB has been clearly shown to be tumorigenic in rats, hamsters and mice following chronic administration in the diet. Increases in tumors were seen in the liver and kidney as well as the adrenal, parathyroid and thyroid glands. To date, the mechanisms underlying carcinogenesis in these organs remain unclear. As described above, several theories have been proposed to explain the basis for certain tumors induced by HCB. While these theories merit further study, none of them has been sufficiently well tested or established at this time for us to recommend that a non-linear dose-response approach be used to estimate risks at low exposure levels. Given the current mechanistic uncertainty, we have used a linear approach to extrapolate carcinogenic risks from high to low doses.

2.1.8 Contamination of HCB in Thailand

Before being banned in 1980, many commercial and industrial activities in Thailand both intentionally and unintentionally involved with HCB and/or its byproducts. The wastes from these high utilizations have entered into the environment leading to the HCB contamination as it is considered as one of POPs. Boonyatumanond et al. (2008) investigated the concentration of the POPs in seawater and fish samples from many provinces along the Gulf of Thailand, i.e., Trat, Rayong, Chanthaburi, Chon Buri, Samut Prakan, Samut Songkharm, Chumphon, Phetchaburi, Prachuab Khiri Khan, Nakhon Si Thammarat, and Surat Thani, to examine the accumulation of POPs including HCB. HCB was detected in two fish samples at 23.7 and 69.8 ng/g fat weights. Moreover, this results indicated that accumulation of HCB in fish samples were lower than the Maximum Residue Limit (MRL) for aquatic animals as recommended by the Ministry of Public Health of Thailand. Even though POPs were not detected in seawater samples in the Gulf of Thailand, the result of fish samples indicated that POPs still remain in Thailand environment and accumulated in biota.

2.1.9 Overview of Microbial Mediated Reductive Dechlorination of Dechlorinated Aromatics by Sediment Slurry

The factors shown in the table 2.2 including microbial factors, chemical factors, and physical factors were studied for microbial mediated reductive dechlorination of chlorinated aromatics by sediment slurry.

Table 2.2 Factors involved in reductive dechlorination of chlorinated aromatics in sediment slurry.

Anaerobic microbial populations	Chemical factors	Physical factors
1.Fermentator 2.Acidogenic, Acetogen 3.Sulfate-reducing bacteria (SRBs) 4.Methanogen - Non-dechlorinated - Dechlorinated	1.Organic compounds 2.Growth factor (vitamins) 3.Unknown factor (minerals) 4.Toxic compounds	1.Temperature 2.pH

Among anaerobic microbial populations, the Fermentators can grow under aerobic and anaerobic conditions, when the environment turned into anaerobic condition, they use organic molecules as electron acceptor to produce CO_2 and H_2 and decompose the large molecules into small ones. Therefore, Fermentators is obligated to change the environment to the micro-oxidative condition, in case of Fermentators are efficient. Whenever the environment changed to the micro-oxidative condition, the Acidogens and Acetogens take over the work and keep utilizing the organic compounds to produce more electron donors and sequentially change the environment to strictly anaerobic condition. In the same period, acidogens and Acetogens keep utilizing the organics to smaller molecules those were essential for the growth of sulfate reducing bacteria (SRBs) and Methanogens. However, SRBs will grab electron for producing sulfate, sulfide and hydrogen sulfide, which will inhibit the growth of Methanogen. Even though, the Methanogen still can get electrons if the donors are at large quantity. In the dechlorination test, there are 2 types of Methanogens, one is HCB dechlorinator and the other has no relationship to HCB dechlorination. Both two types of anaerobes use organic compound and/or carbon dioxide as carbon source and coupled with electrons to produce methane. Therefore, the SRBs and non-dechlorinating Methanogens could

compete with the HCB dechlorinator for the carbon source and electron source, and probably inhibit the dechlorination of HCB.

2.1.10 Pathways of HCB Dechlorination

Chlorinated benzenes are generally believed to be recalcitrant to microbial attack in aerobic condition. The anaerobic degradation pathway of HCB starts with a series of reductive dehalogenation steps. Anaerobic process that transform these compounds produce dehalogenated compounds that are generally less toxic, less likely to bioaccumulation and more susceptible to further microbial attack, especially by aerobic microorganism (Sim et al., 1990). Mohn and Tiedje (1992) indicated that microbial dechlorination entails the action of both an electron acceptor and donor; specifically, when the chlorine atom of the aryl compound accepts an electron and be taken off from the molecule in return and hydrogen was used as the electron donor. This class of compounds is representative of important environmental pollutants (in addition to the chlorobenzenes) such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls which also was subject to dehalogenation (Fathepure et al., 1988). The pathways in dechlorination of HCB are shown in Figure 2.2. Several other studies such as those of Mohn and Tiedje (1992) and Beurskens, et al. (1994) found that HCB dechlorination followed the major pathway to 1,3,5-TCB as proposed by Fathepure et al. (1988) in Figure 2.2. Dechlorination of 1,3,5-TCB to 1,3-DCB is the least attractive step of all reactions. Nonetheless, Jacobus, et al. (1994) reported the HCB dechlorination via QCB, 1,2,3,4-TeCB, 1,2,3- and 1,2,4-TCB, 1,2- and 1,4-DCB to MCB. On the basis of the sequence of dechlorination intermediates from HCB and 1,2,3,5-TeCB, it appears that the HCB was dechlorinated via two routes as shown in Figure 2.2, one leading to the formation of stable 1,3,5-TCB and the other to the formation of DCBs. There are several potential explanations. One is that there may be two populations, each using a different pathway. The second is that the products reflect the distribution of reactive ring intermediates in which chlorine atom that is between other chlorines is lost most readily, and once there are no adjacent chlorines, e.g., 1,3,5-TCB, dechlorination ceases (Fathepure et al., 1988). Berurskens, et al. (1994) reported CB dechlorinating anaerobic microbial consortium was obtained by selective enrichment with HCB and lactate from a freshwater sediment sample that originated from an area with proven in situ HCB dechlorination. The consortium was used to

determine the dechlorination under methanogenic condition. The dechlorinating activity was restricted to benzene with at least three adjacent chlorines, except for a relative slow transformation of 1,2,4,5-TeCB to 1,2,4-TCB. Optimal temperature for dechlorination was around 30°C. Dechlorination of CBs was performed by the activity of a group of microorganisms in which some bacteria played the role of producing hydrogen while others provided the nutrients. But the critical step, in which two electrons with one proton were received and one chloride ion was released for CB molecules, was considered to be achieved by a distinct enzyme, a coenzyme or cofactor, produced by a specific bacterium (Chen et al., 2000). Holliger, et al. (1992) used lactate, glucose, ethanol or isopropanol as the electron donor. A stable consortium obtained by transferring on lactate as the energy and carbon source in the presence of 1,2,3-TCB dechlorinated this isomer stoichiometrically to 1,3- DCB. Lactate, ethanol and hydrogen appeared to be the best substrates. Optimal temperature and pH for dechlorination were 30°C and 7.2, respectively. HCB and QCB were dechlorinated to 1,3,5-TCB and minor amounts of 1,2,4-TCB. 1,3,5-TCB was the sole product formed from 1,2,3,5-TeCB, while 1,2,3,4-TeCB and 1,2,4,5-TeCB were converted to 1,2,4-TCB. Pavlostathis and Prytula (2000) developed a model based on Michaelis-Menten principle describe the batch sequential reductive dechlorination of HCB and other PCB congeners. Michaelis-Menten kinetics is recommended over first-order kinetics for the modeling of the reductive dechlorination of CBs. The predominant sequential HCB dechlorination pathway was HCB to QCB to 1,2,3,5-TeCB to 1,3,5-TCB to 1,3-DCB. Very low levels of 1,2,4,5-TeCB, 1,2,4-TCB and 1,4-DCB were also detected.

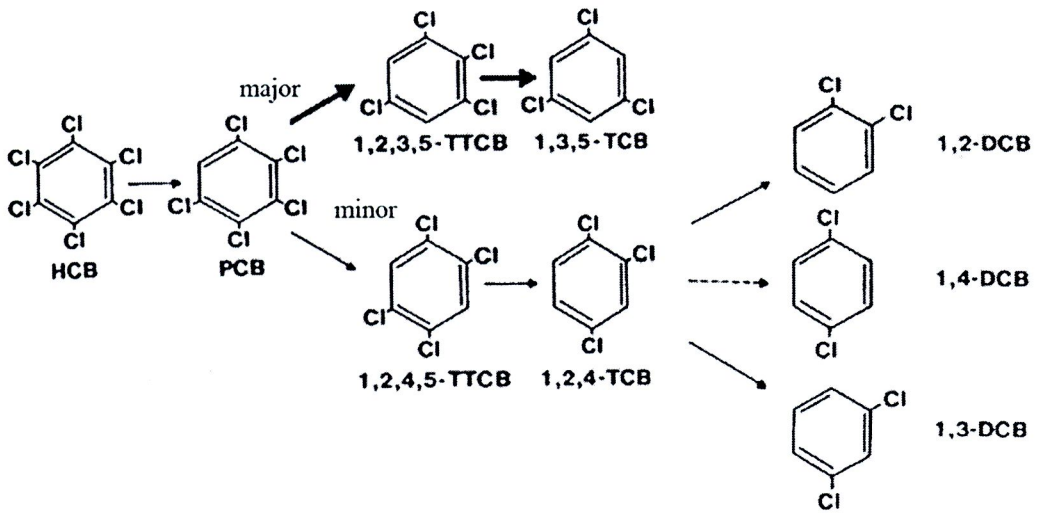

Figure 2.2 Proposed pathway for HCB dechlorination by an anaerobic microbial community (Fathepure et al., 1988).

2.1.11 Environmental Loss Processes

Although HCB is very persistent, it is dose degrades at a low rate in all environmental compartments. For modeling purposes, HCB was assigned approximate half-lives of 17000 hours (1.9 years) in air and 55000 hours (6.3 years) in water and sediment by Mackay, et al. (1992). Ballschmiter and Wittlinger (1991) estimate the net residence time of HCB in air is significantly less than one year, and is based on physical translocation and not on chemical transformation. Loss processes from the environment are discussed in more details for various environmental compartments in the following sections.

2.1.11.1 Degradation in Air

Atmospheric degradation of HCB is extremely slow, and is not an efficient removal process. HCB may be removed from the atmosphere by photolysis and by chemical reaction with hydroxyl (•OH) radicals. Howard (1991) estimated a half-life of HCB in air due to photooxidation ranging from 156 days to 4.2 years. Wanie and Mackay (1995) predicted that the degradation half-life of HCB varied among difference regions, with half-lives of 0.63 years (230 days) in tropical/subtropical region, 1.94 years (708 days) in temperate/ boreal regions, and 6.28 years (2292 days) in polar regions (Parlar, 1978).

2.1.11.2 Degradation in Water

HCB may be removed from water by photolysis, but at a very low rate, with a half-life of about 70 days (Mill and Haag, 1986). HCB in aqueous solution is degraded into pentachlorophenol, which is then degraded further. However, HCB in the water column rapidly sorbs to particulate matters, making it unavailable for photolysis (Schauerte et al., 1982). Thus, photolysis is not expected to be an important fate process. A half-life ranging from 2.7 to 5.7 years in surface water and 5.3 to 11.4 years in ground water has been suggested, based on unacclimated aqueous aerobic biodegradation (Mackay et al., 1992; Howard, 1991).

2.1.11.3 Degradation in Soils

HCB is persistent in soil because it is strongly sorbed to soil organic matter, which gives it a low bioavailability (Beurskens et al., 1994), and due to its completely

substituted nature. The half-life for residence of HCB in soil has been estimated to be 970-2100 days (Griffin and Chou, 1981), with the major loss process from soil at the surface being volatilization. In a study of treated soil stored under aerobic and anaerobic conditions in covered containers to retard volatilization, no detectable loss of HCB occurred over the one-year experiment (Isensee et al., 1976). Aerobic and anaerobic biodegradation are the major means removal at lower soil depths with half-lives of 2.7-5.7 years (Beck and Hansen, 1974) and 10.6-22.9 years (Howard, 1991), respectively. A major problem with these data is that measured 'disappearance' from soils includes both volatilization and degradation. Whereas biodegradation completely removes HCB from the environment, volatilization leads to continued existence of HCB in a different environment compartment. Brahushi, et al. (2004) studied the anaerobic biodegradation of HCB in an arable soil. Several dechlorination pathways were detected; however the main HCB transformation pathway was HCB → QCB → 1,2,3,5-TeCB → 1,3,5-TCB → 1,3-DCB, with 1,3,5- TCB as the main intermediate dechlorination product. The other TeCB-, TCB- and DCB- isomers were also detected in low amounts, showing the presence of more than one dechlorination pathway. Sulphate-reducing or nitrate-reducing microorganisms rather than methanogenic bacteria were implicated as the dechlorinating organisms (Rosenbrock et al., 1997; Brahushi et al., 2004) Bosma, et al. (1988) suggested that complete degradation of HCB in soil or sediment could not occur because as the bacteria breakdown HCB and the availability of further HCB was reduced by mass transfer from the organic matter, a threshold concentration was reached below which no biotransformation was possible. Initial degradation rates were directly related to the aqueous concentration according to the Michaelis-Menten relationship (Pavlostathis and Pyrtula, 2000). Subsequently, retarded desorption occurred, and the effective diffusivity of HCB (mainly controlled by diffusion in OM) controlled the degradation progress. A point was then reached when the 'available' HCB concentration fell below that necessary to support the microbial population feeding on it.

2.1.11.4 Degradation in Sediment

Using fugacity modeling and published reaction rate, Mackay, et al. (1992) suggested that the half-life of HCB was greater than 6 years. Beurskens, et al. (1994) investigated microbial degradation of HCB in the sediment of the Rhine River. They found that significant anaerobic dechlorination had occurred, with up to 80% of the HCB that had

been deposited in the 1970s having been dechlorinated by 1988. They calculated a maximum half-life of HCB in sediment to be 7 years. HCB was transformed to the less toxic and more mobile 1,3,5-TCB, 1,2-DCB and 1,3-DCB. Chang, et al. (1997) investigated the anaerobic dechlorination pathway under laboratory conditions. Dechlorination was strongest in methanogenic conditions, followed by sulphate reducing conditions, but did not occur under denitrifying conditions. Biotransformation occurred along the metabolic decay route: HCB → QCB → 1,2,3,5-TeCB → 1,3,5-TCB + 1,2,4-TCB → 1,3-DCB. Zhao, et al. (2003) also investigated the anaerobic degradation of HCB in sediments, and observed a degradation rate of 0.035 month-1, and increased to 0.088 month-1 when extra organic carbon was added to the sediment. These values correspond to haft-lives of 1.7 and 0.7 years, respectively. A wide range of degradation rates have been reported in other studies, depending on experimental set- p, i.e., 0.110 d-1 (Susarla et al., 1997), 0.021 d-1(Jackson and Pardue, 1998), 0.0256 d-1 (Masunaga et al., 1996) and 0.0022 d-1 (Prytula and Pavlostathis, 1996). These correspond to half-lives of 6.3 days, 33 days, 27 days and 315 days, respectively, all of which were considerably lower than the 6.3 year estimated by Mackay, et al. (1992). HCB has also been shown to degrade anaerobically in sewage sludge, with 1,3,5- TCB as the main product following a similar pathway to that reported by Fatherpure, et al. (1988). This process was examined in detail by Yuan, et al. (1999). Biotransformation occurred along a slightly different pathway of HCB → QCB → 1,2,3,4-TeCB + 1,2,3,5- TeCB → 1,3,5-TCB + 1,2,3-TCB + 1,2,4-TCB → 1,2-DCB + 1,4-DCB. This means that TCB dechlorinated further to DCB, contrary to the finding of Fatherpure et al. (1988). Highest-to-lowest dechlorination rates under three reducing conditions were reported as methanogenic conditions (0.30 mg/l/day), sulphate-reducing conditions (0.23 mg/l/day), and denitrifying condition (0.08 mg/l/day), respectively. Watanabe, et al. (1986) examined the presence of hexabromobenzene and other brominated benzenes in sediments collected at various sites from rivers and estuaries of Osaka, Japan. The study indicated the presence of 1,3,5 - tribromobenzene, 1,2,4,5- tetrabromobenzene, and pentabromobenzene in the sediment; they appeared to be the decomposition products of hexachlorobenzene in the environment. The similarity of products in the two studies provides further evidence for the proposed pathway of HCB dechlorination and suggests that a similar mechanism is possible for the bromine atom removal.

2.1.12 Anaerobic Degradation Process

The biological conversion of the organic matter can be divided in three steps (see in Figure. 2.3). The first step in the process involves the enzyme-mediated transformation (hydrolysis) of higher-molecular-mass compounds into compounds suitable for use as a source of energy and cell carbon. One group of organisms is responsible for hydrolyzing organic polymers and lipids to basic structural building blocks such as monosaccharides, amino acids, and related compounds.

The second step (acidogenesis) involves the bacterial conversion of the intermediate compounds. Group of anaerobic bacteria ferment the breakdown products to simple organic acids, the most common of which in anaerobic digester is acetic acid. The group of microorganisms, described as nonmethanogenic, consists of facultative and obligate anaerobic bacteria. Collectively, these microorganisms are often identified in the literature as "acidogen," or "acid formers."

The third step (methanogenesis) involves the bacterial conversion of the intermediate compounds formed in the second step into simpler end products, principally methane and carbon dioxide. Some microorganisms can convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide. The bacteria responsible for this conversion are strict anaerobes and are called "methanogenic bacteria". They are identified as "methanogens," or "methane formers." It is important to note that methane bacteria can only use a limited number of substrates for the formation of methane. Currently, it is known that methanogens use the following substrates: CO2+H2, formate, acetate, methanol, methylamines, and carbon monoxide for their growth. The methanogens are able to utilize the hydrogen produced by the acidogens because of their efficient hydrogenase.

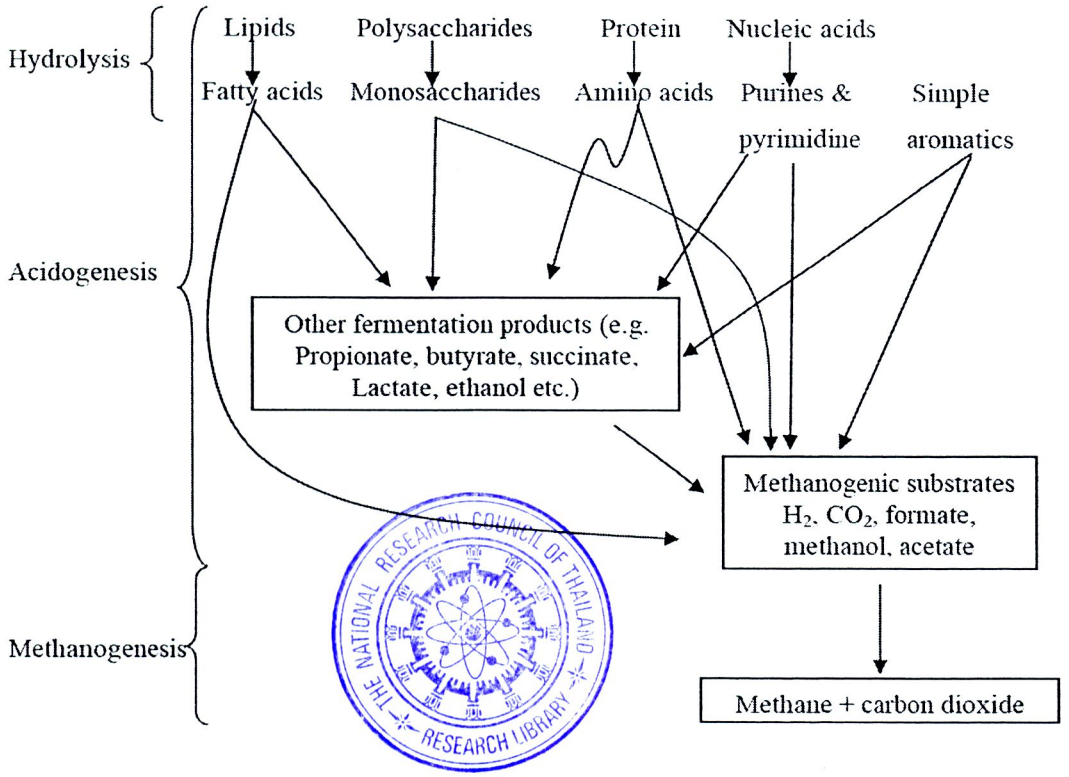

Figure 2.3 Schematic diagram of the patterns of carbon flow in anaerobic digestion (Tchobanoglous and Burton, 1991)

2.2 Literature Reviews

Chen et al. (2001) studied the chlorobenzenes (CBs) and polychlorinated biphenyls (PCBs) dechlorination ability of the untamed microorganisms under anaerobic condition to investigate the relationship between the reaction heat and chromatographic properties for the reductive dechlorination of CBs and PCBs. The culture media were prepared from sludge, river water and a synthetic medium. The results showed that HCB was degraded mainly to 1,3,5-TCB and little to 1,2,4-TCB in both sludge medium and inoculated synthetic medium, but no degradation occurred in the sterile control. Nevertheless, the dechlorination products, 1,3,5-TCB and 1,3-DCB, were not continuously dechlorinated after 76 days of incubation. It implied that the mixed culture did not possess the ability to dechlorinate 1,3,5-TCB. For PCBs dechlorination, no degradation product was found both in sterile control and in inoculated river water medium, but was found in the inoculated synthetic medium. The dechlorinations of CB and PCB congeners were more likely to occur when larger amounts of energy were released and greater Δln RRT value between the parent congener and the daughter product was observed. Using MNDO methodology and GC-ECD techniques were

performed in this study to compute theoretical dechlorination reaction heats and estimate chromatographic data of CB and PCB congeners, respectively.

Chen et al. (2002) studied the capability of microbial to HCB dechlorination under various conditions. The ability to HCB dechlorination was induced by enrichment with yeast extract and inoculation with HCB-acclimated culture. Lactate addition or replacement of yeast extract by lactate did not enhance the dechlorination potential.

In the year of 2004, Brahushi, et al. proceeds in researching the reductive dechlorination and behaviour of ^{14}C-hexachlorobenzene (HCB) by inducing the native anaerobic microbial communities in the saturated soil with water. In this study, methane was detected after four weeks of incubation in all treatments and the highest production rates were observed when wheat and lucerne straw were added to the soil. These results suggested that production of methane was related strongly with the amount of carbon sources in the soil. Interestingly, they also found that additional organic substances (wheat and lucerne straw) considerably delayed and reduced the dechlorination process even though they promoted the activity of methanogenic bacteria. Several dechlorination products were detected with 1,3,5-TCB as the main intermediate dechlorination product, while TeCB-, TCB- and DCB-isomers were also detected in low amounts indicating that there were more than one dechlorination pathway for HCB.

Hirano, et al. (2007) investigated the anaerobic biodegradation potential of HCB in sediment samples collected from three different sites along the Kamogawa River in Japan. HCB was detected with a concentration range of 0.30 - 0.92 ng/g.d.w. in all three sediments. The sterilized control samples were tested. They found that biodegradation of HCB in all three sediments started immediately with the start of the experiment without lag period, which was much faster than that in the sterile control. At the end of 20-week anaerobic incubation in the dark at 30 °C, HCB degradation efficiencies ranged from 47.6% to 59.4%. These results strongly indicate that the degradation of HCB in the river sediment is due to microbial action. Sediment with rich organic content and contaminated with HCB was observed to have high biodegradation rates for HCB more than other pollutants. Moreover, they also found that biodegradation HCB was accompanied by obvious methane generation and drop of oxidation-reduction potential (ORP).

Zhang, et al. (2008) studied of temporal changes in the distribution of exogenous HCB among different soil organic matter fractions under sterile and non-sterile conditions. Soil samples also have different soil water contents and different concentrations of added Cu^{2+}. The results revealed that microbial activity accelerated the mass transfer, while the addition of Cu^{2+} slowed it down. The HCB transfer rate decreased as the soil moisture increased from 1.9% to 60%, but increased when soil moisture increased further to 90%.

In 2007, Winchell and Novak, investigated the effect of biostimulation (supplement of H_2 via elemental iron (Fe^0)) and bioaugmentation (amend of PCB-dechlorinating enrichment culture) spiked with 2345-CBp. Insignificantly dechlorination of 2345-CBp were occurred in both sediment from Raisin River (PCBs contaminated site) and Duluth Harbor (non PCBs contaminated site) under stimulating condition. However, the extensive dechlorination was observed after augmenting microcosms, whereas these microcosms was enriched and grown on acetate (20 mM) under a headspace of 3% H_2 to 97% N_2.

Anotai et al. (2010) studied hexachlorobenzene (HCB) and 1,3,5 trichlorobenzene (1,3,5-TCB) the sediments from the Hua-Lum-Poo Canal located in Samut Prakarn, a province of Thailand. This study was devoted to investigate the reductive dechlorination of HCB by the anaerobic microorganisms in canal sediments. The results, it was shown that after 7 days of pre-anoxic and enriched treatment, canal microbes were able to develop HCB dechlorination activity, and completely dechlorinate HCB within 6 weeks. This indicates that the HCB dechlorinated microorganisms were prevalent in the sediments along the Hua Lum Poo Canal, and possibly remove HCB contamination without any additional nutrient.

In the same year, Chen et al. researched the indigenous microbes from the sediments, could dechlorinate HCB effectively without any acclimation and supplemental nourishment. Temperature seriously affected the HCB-dechlorination: within the measured 15–45 °C span, the optimum range was between 30 and 35 °C. Sulfate-reducing bacteria (SRB), denitrifiers, and acetogens might not be directly involved in the HCB dechlorination. However, the SRB retarded subsequent dechlorination of pentachlorobenzene to tetra- and trichlorobenzenes. Some vancomycin-resistant gram-

positive bacteria and methanogens were most likely to be the HCB-dechlorinators. The dechlorination followed the Michaelis–Menten behavior with the k'm and KHCB between 0.45–0.73 mgL−1 day−1 and 3.2–17.2mgL−1, respectively. These findings suggest a potential HCB treatment and cleanup for wastewater and contaminated site.

Sudjarid et al. (2011) studied dechlorination ability of indigenous microorganisms from seven stream sediments collected around Bangkok and its vicinity in Thailand and was tested by amended with seven polychlorinated biphenyl congeners (PCBs) including 2,3,4-chlorobiphenyl (234-CBp), 22'5-CBp, 24'5-CBp, 22'35'-CBp, 22'45-CBp, 23'44'5-CBp, and 22'34'5'6-CBp. In biostimulation experiments, Hua Lum Poo Canal (HLP) nutrient-strengthened sediment slurry was amended by yeast extract, mineral nutrients, acetate, lactate, and pyruvate, but no significant enhancement was found for 234-CBp dechlorination. It implied that the present substrates and nutrients at this site were sufficient to sustain the activity of 234-CBp dechlorination consortium. In bioaugmentation experiments, sediment slurry from HLP that possessed higher dechlorination potential was introduced to five less effective sediment slurries for 234-CBp dechlorination tests. Results indicated a significant acceleration to those important slurries, and revealed a promising application for the in-situ remediation of PCB contamination in Thailand that might be expanded to worldwide application.

In 2011, Sudjarid et al. evaluated hexachlorobenzene (HCB) dechlorination in pre-treated sediment-water slurries and untreated fresh sediment water slurries. Sediment and water samples were collected from 5 different sites along the Hua-Lum- Poo Canal in Samut Prakarn Province of Thailand. In HCB dechlorination experiments of pre-treated slurries, a variety of natural sediment-water slurries were used as sole cultural media without any supplement and extra nutrient. Sediment-water slurries were prepared in different methods, including the sediment to water ratio 1:2 and 1:4 with or without sterilization, with or without filtration, and with 3 or 7 days of pre-anoxic treatments. The results showed that HCB was dechlorinated more effectively in the sediment to water ratio of 1:2 than in the ratio of 1:4. In the sterilization bottles without inoculation, dechlorination did not happen. With inoculation, HCB dechlorination was observed both in the filtrated and non-filtrated sediment slurry bottles. In addition, HCB dechlorination was better in slurries with 7 days of pre-anoxic treatment than those with 3 days of treatment. For experiments of non-treated fresh slurries, the sediment-water

slurries were prepared with sediment to water in the ratio of 1:1 and 1:2. Without any inoculation and extra nutrient put into these bottles, most of the fresh slurries completed HCB dechlorination in a remarkably speed within 8 weeks. The results suggested that the indigenous microbes of the canal were active in HCB dechlorination and also showed the possibility of natural attenuation and bioremediation of HCB-contamination in these sites.